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EXAMINER	
SALMON, KATHERINE D	
ART UNIT	**PAPER NUMBER**
1634	

DATE MAILED: 04/21/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 10/782,646	Applicant(s) HYLDIG-NIELSEN ET AL.	
	Examiner Katherine Salmon	Art Unit 1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 March 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-49* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-49* is/are rejected.

7)☒ Claim(s) *10* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☒ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *12/15/2005*.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application (PTO-152)

6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election without traverse of Group I, Claims 1-49 in the reply filed on 3/14/2006 is acknowledged.

2. Claims 50-51 have been canceled.

3. Claims 1-49 are pending.

Oath/Declaration

4. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

> The oath or declaration is defective because:
> It does not identify the mailing address of each inventor. A mailing address is an address at which an inventor customarily receives his or her mail and may be either a home or business address. The mailing address should include the ZIP Code designation. The mailing address may be provided in an application data sheet or a supplemental oath or declaration. See 37 CFR 1.63(c) and 37 CFR 1.76.

Claim Objections

5. Claim 10 objected to because of the following informalities: a period should be placed at the end of the claim. Appropriate correction is required.

Claim Rejections - 35 USC § 102

> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

6. Claims 1-4, 6, 9-10, 26-28, 31-33, 36-37, 39-44, and 46-49 are rejected under 35 U.S.C. 102(b) as being anticipated by Wittwer et al. (US Patent 6,140,054 October 31, 2000).

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. With regard to Claims 1, 10, 36, 46-49, Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

With regard to Claim 2, Wittwer teaches a method of using and monitoring fluorescent probes (Column 7 lines 60-61). With regard to Claim 3, Wittwer et al. teaches that multiple sets of FRET pairs can be labeled with different fluorescent resonance energy transfer pairs so that the sets of FRET oligonucleotide pairs can be

distinguished from one another based on the distinguishable emission spectra (Column 12, lines 57-61).

With regard to Claim 4, Wittwer et al. teaches FRET oligonucleotide pairs having different melting temperatures for each of the FRET oligonucleotide pair is preferred (Column 13, lines 9-11).

With regard to Claims 6 and 9, Wittwer et al. teaches different sets of FRET oligonucleotide pairs can be labeled with the same fluorescent resonance transfer pair, allowing for monitoring at a single emission wavelength (Column 12 lines 49-53).

With regard to Claims 26-28 and 31-33, Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). Therefore it is inherent in the teaching of Wittwer et al. the melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

With regard to Claim 37, Wittwer et al. teaches a method in which the polynucleotide sample is an amplified product (Column 4, lines 20-25).

With regard to Claims 39-41, Wittwer et al. teaches multiple loci of a target nucleic acid sequence can be analyzed (column 12 lines 41-42). With regard to Claim 42 and 44, Wittwer et al. teaches co-amplifying two or more separate regions of nucleic acid using at least two sets of PCR primers and at least two sets of FRET oligonucleotide pairs as probes to simultaneously genotype the separate regions by analyzing the melting temperature of the sets of FRET oligonucleotide pairs (Column 16 lines 11-20).

With regard to Claim 43, Wittwer et al. teaches the melting peaks of each set of probes must be distinguishable from the next set of probes (Column 15 lines 5-10). Wittwer et al. teaches to distinguish the melting point peaks of two sets of probes the probes are designed so the melting temperature of each set of probes is different from the melting temperature of the other set of probes (Column 15, lines 5-10). Wittwer et al. teaches that to differentiate amplification products the melting temperature can be separated by less than 2°C (column 16, lines 5-10).

Claim Rejections - 35 USC § 103

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 11-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tsourkas et al. (Nucleic acid research 2002 Vol. 30 p. 5168).

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor

(signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39). Wittwer et al., however, does not teach the use of self-indicating signal probes such as hairpin probes.

Tsourkas et al. teaches a method of using methyl labeled molecular beacons to increase hybridization results (Abstract). With regard to Claims 11 and 12, Tsourkas teaches molecular beacons is a stem-loop confirmation (hairpin) that is quenched until it opens when hybridized to a target (2^{nd} column 1^{st} paragraph). With regard to Claims 13-15, Tsourkas et al. teaches that false-positive signals are fluorescence signals induced by the opening of molecular beacons due to nucleases (p. 5168 2^{nd} column 2^{nd} paragraph). Tsourkas et al. teaches that nuclease degradation can be reduced by adding phosphorothioate, PNA, and 2'-O-methyl (p. 5168 2^{nd} column 2^{nd} paragraph). Tsourkas et al. teaches that false-positives can be further reduced by using two molecule beacons that bind to adjacent regions on a target molecule and generate positive signals via FRET (p. 5168 2^{nd} column 2^{nd} paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use hairpin probes as signal probes as taught by Tsourkas et al. The ordinary artisan would

have been motivated to modify the method of Wittwer et al. because Tsourkas et al. teaches tem-loop structure of a molecular beacon improves the specificity of target discrimination compared with linear probes (p. 5168 2nd column 1st paragraph). Tsourkas et al. teaches the competing reaction between hairpin formation and target hybridization increases the sensitivity of detecting a SNP between probe and target sequences and thus enables molecular beacons to differentiated between wild-type and mutants targets better than linear probes (p. 5168 2nd column 1st paragraph).

10. Claims 11-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Sokol et al. (PNAS 1998 Vol 95 p. 11538)

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor

(signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach the use of self-indicating signal probes such as hairpin probes.

Sokol et al. teaches a method of real time detection of DNA and RNA hybridization in living cells (Abstract). With regard to Claims 12-13, Sokol et al. teaches the use of Molecular beacons (hairpin probes) as the reporter (signal) oligonucleotide (abstract). The instant specification defines “self-indicating probes” as a signal probe that produces little or no detectable signal when free in solution (p. 7-8 paragraph 80). Sokol et al. teaches molecular beacons when in the stem loop confirmation (not hybridized so therefore in solution) produce no signal emission (abstract). With regard to Claim 13, Sokol et al. teaches molecular beacons provide greater nuclease resistance (p. 11541 last sentence). With regard to Claims 14-15, Sokol et al. teaches using DNA molecular beacons (p. 11538 last paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use molecular beacons as signal probes as taught by Sokol et al. The ordinary artisan

would have been motivated to modify the method of Wittwer et al. because Sokol et al. teaches a potential problem with FRET detection is that the loss of fluorescence might not mean conclusively that hybridization has taken place therefore the use of molecular beacons allows for direct demonstration that duplex formation took place and FRET was suppressed (p. 11538 2nd column first full paragraph).

11. Claims 11, 16-22, and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Gaylord et al. (PNAS 2002 Vol 99 p. 10954).

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a

lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach the use of self-indicating signal probes.

Gaylord et al. teaches a method of using PNAs as probes to detect point mutations in cystic fibrosis. With regard to Claims 11, 16, and 18-20, Gaylord et al. teaches the use of PNA probes to detect point mutations (abstract). With regard to Claim 17, Gaylord et al. teaches that PNAs are less susceptible to biological degradation by nucleases (p. 10954 2nd column 1st paragraph).

With regard to Claims 21-23, Gaylord et al. teaches the use of a cationic water-soluble CP poly(9,9 bis(6'N,N,N-trimethylammonium)-hexyl)-fluorene phenylene) containing iodide counteranions (hexium iodide) (p. 10954 2nd column 2nd full paragraph).

With regard to Claim 38, Gaylord et al. teaches a method using single-stranded DNA in FRET analysis (p. 10954 2nd Column 1st full paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use PNA probes as taught by Gaylord et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Gaylord et al. teaches PNA

probes form complexes quickly and are tighter and more specific than analogous DNA/DNA complexes (p. 10954 2nd column 1st sentence).

12. Claims 11, 16-20, and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Kubista et al (US Patent 6329144 December 11, 2001).

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al.

teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach the use of self-indicating signal probes.

Kubista et al. teaches a method of using a probe for the detecting nucleic acids having a particular sequence (Abstract). With regard to Claims 11, 16, and 18-20, Kubista et al. teaches the use of PNA based probes. The instant specification defines "self-indicating probes" as a signal probe that produces little or no detectable signal when free in solution (p. 7-8 paragraph 80). Kubista et al. teaches a method that has minimal luminescence free in solution and strong luminescence bound to nucleic acids (Column 6 lines 43-45). With regard to Claim 17, Kubista et al. teaches that PNA based probes are resistant to nucleases (Column 9, line 6). With regard to Claim 38, Kubista et al. teaches the PNA forms a more rigid duplex with single stranded than double stranded nucleic acids which more effectively restricts the internal motion in the bound reporter and increases fluorescence signal (Column 10 lines 63-65).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use PNA probes as taught by Kubista et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Kubista et al. teaches PNA based probes obtain an higher signal than nucleic acid based probes when hybridized to the target strand (Column 18, lines 56-57). The ordinary artisan would use a method, which had the highest measurable signal in order to be able to determine signal versus background signal more efficiently.

13. Claims 11 and 21-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al (US Patent 6277607 August 21, 2001).

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teaches using an intercalating dye.

Tyagi et al. teaches a method of nucleic acid amplification in which primers are in hairpin structures (abstract). With regard to Claim 11, Tyagi et al. teaches the use of hairpin primers, which can monitor the amplification reactions by florescence (Column 6, lines 52-53). With regard to Claims 21-23, Tyagi et al. teaches that the assays can be detected using intercalating dyes (Column 4, lines 6-10). Tyagi et al. teaches that one type of intercalating dye that can be used is SYBR Green (Column 11, lines 39-41).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use hairpin probes and intercalating dye as taught by Tyagi et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Tyagi et al. teaches the use of hairpin probes reduces the probability of primer dimers, allows for real-time detection of the amplification product for accurate quantification of the initial number of target sequences in a sample (Column 7, lines 1-15). Tyagi et al. teaches that the use of an intercalating dye allows for the monitoring of each reaction (Column 11, lines 35-40).

14. Claims 24-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Singer et al. (US Patent 6,323,337 November 27, 2001).

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of

sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach the use a fluorescent minor groove binding dye.

Singer et al. teaches a method to label oligonucleotides (abstract). With regard to Claims 24 and 25, Singer et al. teaches the use of nucleic acid stain such as Hoechst 33342, Hoechst 34580, and DAPI (Column 14 lines 19-25).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use

intercalating dye as taught by Singer et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Singer et al. teaches dyes, which are useful in the combination of quenching oligonucleotides to minimize the fluorescent signal from selected oligonucleotides (Column 16, lines19-22).

15. Claim 45 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Schalasta et al. (Infection 2000 Vol 28 p85).

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a

lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach using the method for virus genotyping.

With regard to Claim 45, Schalasta et al. teaches a method of genotyping type-specific HPV Type 1 and Type 2 using fluorescence Melting Curve analysis (Abstract).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use in genotyping viruses as taught by Schalasta et al. The ordinary artisan would have been motivated to use the method of Wittwer et al. because Schalasta et al. teaches the use of FRET based melting curve analysis provides a rapid diagnosis, high sensitivity, and specificity (abstract).

16. Claims 5 and 7-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Caplin et al. (Biochemical 1999 No. 1 p. 5)

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of

sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

With regard to Claim 8, Wittwer et al. teaches to distinguish the melting point peaks of two sets of probes the probes are designed so the melting temperature of each set of probes is different from the melting temperature of the other set of probes (Column 15, lines 5-10). Wittwer et al. teaches that to differentiate amplification products the melting temperature can be separated by less than 2°C (column 16, lines 5-10).

Wittwer et al., however, does not teach the melting temperature differences between the probes.

Caplin et al. teaches a method of direct mutation detection (abstract). With regard to Claims 5 and 7, Caplin et al. teaches for mutation detection, the best melting curves are obtained when the difference between the probes (quencher and signal) is between 5-10°C (p. 6 1st paragraph). With regard to Claim 8, Caplin teaches that for detection the melting temperature of hybridization probes should be within 2°C (p. 6 1st paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use the melting temperature differences between probes as taught by Caplin et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Caplin et al. teaches the best melting curves are obtained with the specific temperature difference of 5-10°C (p. 6 1st paragraph). The ordinary artisan would be motivated to create the second signal probe at least 7 degrees less than the first signal probe to ensure that the second set could be detected (5 degrees from the quencher and 2 degrees below the other hybridized probe).

17. Claims 29 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic Acids Research 2002 Vol 30 p. e122)

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a

high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). The melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

Wittwer et al., however, does not teach the temperature rate range.

Marras et al. teaches a method of real-time fluorescence using a rapid cycle PCR. Marras et al. teaches that using the 7700 Prism spectroflurometric thermal cycler the temperature was raised in increments of 1°C/minute.

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use the ramping speed as taught by Marras et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Marras et al. teaches the ramping speed acceptable to a commercially produced thermal cycler. The ordinary artisan would want to use the ramping speed that gives the best results for detection of melting temperature.

18. Claims 29 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Elenitoba-Johnson (US Patent 6346386 February 12, 2002)

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of

sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). The melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches

temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

Wittwer et al., however, does not teach the temperature rate range.

Elenitoba-Johnson teaches a method for determining alterations in a nucleic acid using a fluorescent label and melting profiles (Abstract). With regard to Claims 29 and 34, Elenitoba-Johnson teaches a method of performing RT PCR in which the temperature of the sample is raised at a rate slow enough to distinguish between the melting points of the wild type and the fragment of interest (Column 7 lines 5-10). Elenitoba-Johnson teaches a ramping rate of between 0.1 °C/sec to 0.01°C/sec (6°C/min to .6 °C/min) (Column 7, lines 11-20). Elenitoba-Johnson teaches a PCR amplification method in which the probes and target are increased above the melting point of the probes to below the melting point of the probes (Column 10 Example 3).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use the ramping speed as taught by Elenitoba-Johnson et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Elenitoba-Johnson et al. teaches a ramping speed in which it is slow enough to distinguish between the melting temperatures of two sequences (Column 7, lines 1-5).

19. Claims 30 and 35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Witter et al. (named Witter B) (US Patent 6,245,514 June 2001)

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridized to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a

high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). The melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

Wittwer et al., however, does not teach the rate of monitoring the detectable signal.

Wittwer B teaches a method of measuring FRET pairs for detecting the presences of a target analyte (Abstract). Wittwer B teaches measuring fluorescent continuously every 200 msec as a function of temperature (Column 35 lines 29-33).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use the monitoring rate as taught by Wittwer B. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Wittwer B teaches a monitoring rate in which data can be obtained that cannot by measuring fluorescence at each temperature (Column 37, lines 1-5). The ordinary artisan would want to modify the method in order to gain as much information as possible during the monitoring phase in order to make a more precise fluorescence vs. melting temperature curve.

Conclusion

20. No Claims are allowable over the cited prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Katherine Salmon whose telephone number is (571) 272-3316. The examiner can normally be reached on Monday-Friday 8AM-430PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Katherine Salmon 4/17/2006
Katherine Salmon
Examiner
Art Unit 1634

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